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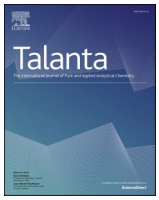

Nanomaterials for optical biosensors in forensic analysis

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ABSTRACT

Biosensors are compact analytical devices capable of transducing a biological interaction event into a measurable signal outcome in real-time. They can provide sensitive and affordable analysis of samples without the need for additional laboratory equipment or complex preparation steps. Biosensors may be beneficial for forensic analysis as they can facilitate large-scale high-throughput, sensitive screening of forensic samples to detect target molecules that are of high evidential value. Nanomaterials are gaining attention as desirable components of biosensors that can enhance detection and signal efficiency. Biosensors that incorporate nanomaterials within their design have been widely reported and developed for medical purposes but are yet to find routine employment within forensic science despite their proven potential. In this article, key examples of the use of nanomaterials within optical biosensors designed for forensic analysis are outlined. Their design and mechanism of detection are both considered throughout, discussing how nanomaterials can enhance the detection of the target analyte. The critical evaluation of the optical biosensors detailed within this review article should help to guide future optical biosensor design via the incorporation of nanomaterials, for not only forensic analysis but alternative analytical fields where such biosensors may prove a valuable addition to current workflows.

1. Introduction

Forensic science can be defined as a branch of analytical science that focuses on the application of scientific principles and knowledge to matters of criminal justice, principally relating to the observation, collection, examination and analysis of physical evidence during the course of an investigation [1]. Forensic science can be further divided into a range of applications, including, but not limited to: biological trace analysis, illicit drug screening, bulk drug analysis, environmental testing, and security and terrorism monitoring. It is a field in which errors or variable results cannot be tolerated and the specificity and sensitivity of the analysis can be critical to the deliverance of justice. Analytical techniques commonly used within forensic science serve the purpose of detecting molecules that can be used as evidence within a case. Currently employed analytical techniques may suffer from certain drawbacks, such as a lack of specificity and sensitivity, requiring technical expertise, being laboratory-based (as opposed to field-deployable) or costly. Biosensors, first reported in 1962 [2], have gained attention in recent years as they have become commercially available tools for a range of analytical fields. Biosensors are compact analytical devices that are capable of transducing a biological interaction event into a measurable signal output in real-time. A biosensor typically consists of a supramolecular complex compromised of two components: a biological sensing element that recognises a specific analyte and a transduction element that produces an output signal upon successful interaction with a target substrate [3,4]. Approximately 85% of the world's market for biosensors are manufactured for blood glucose measurements in patients with diabetes, however recent developments in the field have shown the applicability of biosensors to various fields including forensics [3].

The 'ideal' biosensor for forensic analysis should be sensitive, highly specific, and relatively simple to operate. Additional advantages such as being cost-efficient would also be beneficial due to the high number of samples often processed in forensic analysis. By incorporating a biological sensing element into the biosensor design (e.g. antibodies, aptamers, peptides), a high degree of selectivity can be achieved for a wide range of target analytes. Biosensors within forensic analysis not only have the potential to save time, which can be crucial in fields such as crime scene analysis or drug identification where the results could help to bring an offender to justice quickly, but also could decrease the rate of false positive results encountered [5]. A further advantage of using biosensors for forensic analysis is their ability to be multiplexed, meaning that multiple tests for different targets can be combined into one assay, so samples can be tested for the presence of multiple target

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analytes simultaneously. For example, testing an unidentified suspected drug sample for the presence of several illicit compounds or cutting agents, or a stain at a crime scene for the presence of multiple bodily fluids. Furthermore, the portability of biosensors means that analysis that was once rendered only to laboratory-level use is now capable of being carried out in situ where they may be needed.

Within this review, optical biosensors are identified as an improvement to currently employed field-based analysis techniques for use within forensic analysis as they allow for the direct detection of many target analytes in real-time, with high specificity and sensitivity. Optical biosensors can be defined as analytical devices that contain an integrated optical transducer system. They can produce an optical signal that is proportionate to the concentration of a measured analyte. Optical signals include colourimetry, fluorescence, chemiluminescence and Raman scattering [6]. The produced optical signal has low noise and is often unaffected by external disturbances, so is thought to be advantageous over other physical signals such as electrical or thermal. Optical biosensors have the potential to make an impact within forensic analysis as they are relatively simple to operate in comparison with the likes of electrochemical sensors, which require additional technical knowledge. They also produce clear optical output signals that are easily measured and recorded, which is desirable for a forensic setting where the rapid detection of target analytes is usually required. Personnel conducting portable tests, such as pitch side anti-doping or crime scene stain testing, often do not have specific scientific training, so any optical output signal must be easily seen, recorded and interpreted. Within optical biosensing, two detection methods are most commonly used: fluorescence-based and label-free detection. Fluorescence-based detection can involve either the target or recognition molecules being labelled with fluorescent tags. The occurrence of a fluorescent output signal indicates the presence or absence of target molecules within a testing matrix [7,8]. Within label-free detection, molecules are not labelled or chemically altered in any way and are detected in their natural form. The optical signal is then developed by direct interaction of the target analyte with the transducer [7]. Label-free detection platforms are regarded as being simpler and more cost-effective to perform as opposed to fluorescence-based platforms. Both protocols are commonly used in optical biosensing to give clear, precise information regarding target interaction with the biological sensing moiety, which results in optical biosensors being more versatile than other sensing platforms.

A finite range of commercially available optical biosensors has been developed for forensic analysis. Whilst there is much debate over whether immunoassays are 'biosensors' in the conventional sense of the term, they are the only biosensor form that is commercially used within forensic analysis to the best of our knowledge. Immunoassays, however, such as ELISA (enzyme-linked immunosorbent assay), pose the disadvantage of a high rate of false positives, as well as their lack of quantitative data, which is relied upon in fields such as forensic toxicology [9]. A recently developed commercial kit for the detection of semen, termed RSID™-Semen, has the potential to detect human semen with a limit of detection (LOD) of 1 μL. This lateral flow immunochromatographic strip test functions by detecting semenogelin, a seminal protein, using monoclonal anti-human semenogelin antibodies [10]. The detection principle is based on one of the antibodies being conjugated to colloidal gold and this is then deposited on a pad beneath the sample window. The second antibody is then contained on the test strip, which gives a red colour change to indicate the presence of semenogelin [11]. Despite their promise for sensitive and specific detection of human semen, the high cost of these kits does not warrant their use at all crime scenes [12]. RSID™ kits are also available for a range of biological fluids, such as saliva and blood. Optical biosensors have also been employed for roadside testing of drugs and alcohol. Whilst traditional laboratory-based chromatographic methods remain superior in their quality of sample analysis, the advantages of portability and quick results that biosensors offer mean that novel methods are being more widely incorporated. DrugWipe, a point of care lateral flow immunoassays for the detection of illicit drugs and alcohol, is commercially employed by 42 police forces across the United Kingdom [13]. Using saliva and sweat as the testing matrices, this colourimetric biosensor functions similarly to the aforementioned RSID™ systems. Using drug-specific antibodies conjugated to gold nanoparticles, a positive detection is indicated by the formation of a coloured test line. DrugWipe requires only 10 μL of the sample and has a LOD for benzodiazepines of 5 ng/mL and a LOD for Δ9-tetrahydrocannabinol (THC) of 30 ng/mL. Commercial kits are available for the detection of narcotics such as cannabinoids, opiates, cocaine, benzodiazepines, amphetamines and methamphetamines [14]. Despite these assays providing identification of forensically relevant targets, the drawbacks of false positive and negative rates, the limits of the sample matrix, high cost and lack of quantitative results have driven the need for an optical biosensor-based system that can provide a high-throughput, cost-effective testing of forensic samples.

Throughout the evolution of research associated with optical biosensor design, a wide range of optical platforms, which employ different building blocks, have been formed. The focus of this review will be on those that have incorporated nanomaterials (NMs) into their design using an optical output signal to indicate target detection. NMs traditionally serve as a transduction mechanism and allow for increased sensitivity and lowered LOD due to the interaction of the sensing moiety and NMs [15]. Within the scope of this review article, prominent NMs as well as their synthesis and transduction mechanisms within optical biosensors for forensic analysis are critically evaluated. Commonly used NMs, such as gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), semi-conductor quantum dots (Qdots) and polymer nanoparticles (PNPs), will be reviewed. Their properties and application within forensically relevant optical biosensors will be discussed.

2. Nanomaterials

Nanomaterials are gaining global attention within the medical field, due to their success in drug delivery systems, bioimaging and diagnostics. The nanoscale is broadly defined as the size <100 nm but is sometimes extended to a dimension <1 μm [16]. Therefore, particles that have a size of <100 nm are generally termed 'nanoparticles' (NPs). Widely used NMs can be categorised into four groups: carbon-based, inorganic-based, organic-based and composite-based, but can also be categorised by their properties, shape or size [17]. Carbon-based NMs come in different morphologies such as hollow tubes, ellipsoids or spheres. The unique hybridization properties and sensitivity of carbon's structure can change within its synthesis, allowing for a high level of variation and manipulation that is not yet possible with inorganic NMs. They are widely employed due to their electrical conductivity, high strength, structure, electron affinity, and versatility within analytical fields [18,19]. Inorganic-based NMs consist largely of metal NPs, Qdots, silicon Qdots and upconversion NPs. Their unique optical properties, such as narrow emission bands, have resulted in these NPs often being used to replace traditional fluorophores within biosensing platforms. Due to this advantage, inorganic-based NMs are being used within advanced biosensor probes based on the phenomena of Förster resonance energy transfer (FRET) and quenching, and are becoming widely used within analytical science. However, there are biocompatibility concerns over the use of inorganic-based NMs over the more commonly explored organic-based NMs [20,21]. Organic-based NMs include NMs that are mainly created from organic matter (excluding carbon-based NMs which are large enough to be grouped separately). These NMs utilise non-covalent, weak interactions for their self-assembly. The design of molecules helps to transform the organic NMs into desired structures such as dendrimers, micelles, liposomes and PNPs. The main groups of organic-based NMs are liposomes, micelles, protein/peptide-based and dendrimers. They offer relatively simple routes for the encapsulation of materials and are biodegradable in nature, which has rendered organic-based NMs the most appealing choice

for use in many drug delivery and biomedical systems [22,23]. Composite-based NMs are composed of multiple NMs that are entrapped within a bulk material. This may comprise a combination of soft and hard NMs, two soft NMs, or two hard NMs. Composite-based NMs can be a combination of carbon-based, metal-based, or organic-based NMs with the bulk material being metal, ceramic or polymer. Nanocomposites possess improved properties in comparison to the constituent elements in their un-combined states. They also demonstrate improved mechanical strength, high electrical and thermal conductivity and high resistance to corrosion and wear [24,25]. Example nanomaterials within these four groups are summarised in Table 1. NMs can be synthesised by various methods, however, these methods are commonly divided into two main approaches: bottom-up or top-down synthesis [19]. With bottom-up synthesis techniques, also known as a 'building up' approach, NMs are formed from smaller molecules or atoms [26]. Methods of bottom-up synthesis include atomic layer deposition, metal-organic

Table 1
Four categories of nanomaterials commonly used within optical biosensors: carbon-based, inorganic-based, organic-based and composite-based. Examples of each category are given as well as a description of each NM group.

Class	Nanomaterial	Structure Description	Size	Synthesis	References
Carbon-based	Fullerenes	Unique cage structure containing multiple modification sites. 20 hexagonal and 12 pentagonal rings. Large surface area, for immobilisation of a greater number of recognition moieties.	30-3000 carbon atoms	Laser irradiation of carbon or poly aromatic hydrocarbons, resistive arc heating of graphite or electric arc heating of graphite.	[35,36]
	Carbon Nanotubes	Hollow structures with one or more walls and a nanometre-scale diameter. Sp^2 bonding arrangement, so are one of the strongest fibres. Serve as scaffolds for immobilisation of biomolecules on their surface.	Diameter: 0.4–40 nm	Electric arc discharge, laser evaporation and chemical vapor deposition.	[37,38]
	Carbon Nanofibers	Have only basal graphite planes and edge planes. Structure has potential for surface modification and functionalisation.	Diameter: 0.4–3.0 nm. Length: 2–100 nm	Thermal chemical vapor deposition, plasma enhanced chemical vapor deposition, or electrospinning.	[39,40]
	Graphene	Two-dimensional sheet of sp^2 hybridized carbon atoms packed into a honeycomb lattice. Short carbon bond length of 0.142 nm, making it the thinnest known material with good strength.	Diameter: 1–10 nm. Length: several to hundreds nm	Mechanical cleaving, chemical synthesis, chemical exfoliation, epitaxial growth or thermal chemical vapor deposition.	[41,42, 43]
Inorganic-based	Gold NPs	AuNPs assumed to be quasi-spherical are actually Platonic, Archimedean, or Catalan solids. Display unique optical properties due to their size-dependent electrochemistry, and their high chemical stability.	Diameter: 1–100 nm	Two-phase synthesis of thiolated NPs, the sequestration and reduction of gold salts within dendrimers and controlled growth via the seeded approach.	[44,45]
	Silver NPs	NPs composed of a hollow icosahedral inner shell and a dodecahedral outer shell. They can alter physical, chemical, and biological properties due to their surface-to-volume ratio.	Diameter: 1–100 nm	Evaporation-condensation using a tube furnace at atmospheric pressure, reduction of silver salts via two stages: nucleation and subsequent growth.	[46–48]
	Quantum Dots	Semiconductor crystals comprised of groups II to VI or III to V elements. Have a high luminescence yield and the potential of adjusting emission and absorption wavelengths by altering particle size.	Diameter: 2–10 nm	Top-down synthesis: molecular beam epitaxy, ion implantation, electron beam lithography, or x-ray lithography. Bottom-up synthesis: colloidal Qdots being prepared via self-assembly in solution after chemical reduction.	[49,50]
	Iron-Oxide NPs	Consists of maghemite (γ-Fe_2O_3) and/or magnetite (Fe_3O_4) particles. Possess properties such as superparamagnetism, high surface-to-volume ratio, large surface area, and an easy separation methodology.	Diameter: 1–100 nm	Co-precipitation, thermal decomposition, microemulsions, hydrothermal synthesis, gas-phase deposition, electron beam lithography, pulsed laser ablation, laser induced pyrolysis or combustion.	[51,52]
	Silicon-Quantum Dots	Nanometre-sized particles of crystalline silicon.	Diameter: 1–10 nm	Laser ablation, non-thermal plasma synthesis, electrochemical etching, reduction of silicon halides, thermal destruction of silicon-rich oxides or hydrothermal decomposition of different Si-contained organic precursors.	[53]
Organic-based	Polymer NPs	PNPs include nanospheres and nanocapsules. Nanospheres are matrix particles, so their entire mass is solid. Nanocapsules are vesicular systems where an entrapped substance is confined to an inner cavity, surrounded by a solid shell.	Diameter: 1–1000 nm	Prepared from preformed polymers, or via direct polymerisation of monomers using traditional polymerisation or polyreactions.	[54,55]
	Solid Lipid NPs	Solid matrix structure that makes them stable and often cheaper alternatives to other organic based NPs.	Diameter: 50–1000 nm	High pressure homogenization, emulsion/solvent evaporation, phase inversion and microemulsion.	[56,57]
	Dendrimers	Radially symmetric molecules with a well-defined, homogeneous and monodisperse structure. Consist of a symmetric core, an inner shell and an outer shell.	Diameter: 5–20 nm	Divergent or convergent method. Dendrimers grow outwards from their core by reactions with monomer molecules.	[58]
	Micelles	A collection of amphiphilic surfactant molecules that spontaneously aggregate in water into a spherical vesicle.	Diameter: 5–100 nm	Formed from small molecules with a hydrophilic, polar, or charged 'head' group and a hydrophobic tail, often composed of the hydrocarbon portion of long fatty acids.	[59,60]
Composite-based	Simple Hybrids	A mixture of organic and inorganic materials of submicron-scale dimensions. They combine the benefits of multiple materials resulting in nanocomposites with enhanced properties that cannot be achieved by either element alone.	Diameter: 1–100 nm	Fast mixing process and laminar flow methods.	[61,62]
	Core/Shell Structured	Composed of a core in the centre and a coated outer shell. Can be produced from a range of different materials, such as polymers, inorganics and metals. Shell structure can provide a biocompatible, inert surface for immobilisation, and the nanoparticle core can be polymeric, inorganic or metallic.	Diameter: 5–500 nm	Microfluidic strategies, such as laminar and segmented flow.	[63,64]

decomposition and physical and chemical vapor-phase deposition [27]. The other synthetic route (i.e. top-down) uses a destructive approach for synthesis. The synthesis begins with larger molecules that are decomposed into smaller units that are converted into suitable NPs. Examples of top-down synthesis include mechanical milling and chemical etching [19].

The most commonly utilised NMs for incorporation into optical biosensors are AuNPs, AgNPs, Qdots and PNPs [28]. These NMs have been chosen for incorporation into optical biosensors due to their superior optical properties, long-term stability and ease of synthesis. Whilst these NMs are alike in their optical properties, their modes of detection vary depending on the biosensor in which they are used. For example, AuNPs have a wide range of optical properties, including being a known quencher of fluorescence, so can act within 'turn-off' fluorescence-based biosensors [29], but also display colourimetric properties, meaning they can be used within biosensors where a colour change reaction can be seen via the naked eye [30]. PNPs are also a versatile transducer, as they can be synthesised with a wide range of surface chemistries, as well as different emission spectra. This makes them ideal for use in multiplex analysis, where different emissions can indicate the presence of different target molecules.

One key advantage of incorporating certain NMs, such as AgNPs or Qdots, into a biosensor is that high surface areas allow for an elevated number of recognition units to be attached, resulting in improved sensitivity of the probe. Certain NMs, such as AuNPs, can be utilised for their colourimetric properties that allow a positive target interaction to be identified by a colour change. This colour change is due to gold nanoparticles possessing localised surface plasmon resonance (LSPR) properties with high molar extinction coefficients, which allow them to exhibit size-dependant colour changes that can be detected by the naked eye [31]. Colourimetric assays are highly valued within forensic analysis as they produce a rapid and easily detected signal output. A further advantage of NMs within optical biosensors is the enhancement of stability of the biosensing units and signal output that NMs can produce.

The well-established incorporation of NMs further serves as an advantage of their use within optical biosensors. NMs can be chemically incorporated within biosensor complexes by one of three main methods: non-covalent bonding, covalent bonding, or supramolecular/coordinative interactions. Non-covalent conjugation occurs via electrostatic interactions, entrapment in polymers, van der Waals forces, or π-π stacking, with all methods preserving the properties of the NM and the sensing moiety [32]. Covalent bonding presents an advantage over non-covalent conjugation in terms of stability and reproducibility of the surface functionalisation and also lowers the non-specific physisorption of the biological recognition element to the surface of the NM. Immobilising recognition units via supramolecular or coordinative interactions has been widely accepted as a robust method for conjugating NMs and sensing moieties. These interactions include electrostatic or hydrophobic interactions, α-helical coiled-coil or β-sheet aggregations [33]. The advantage of this route is reversibility, allowing the regeneration of the transducer element. However, the immobilisation of a sensing moiety to the NM may interfere with the binding properties of the moiety meaning that the immobilisation method must be rigorously investigated and optimised to retain the highly specific binding of the moiety to the target [34].

Despite the aforementioned advantages, a noted disadvantage of certain NMs is the potential in vitro and in vivo toxicity [65]. The toxicity of any reagents used within a forensic workflow must be carefully considered. Effects of any toxicity of reagents can have a detrimental impact on not only the sample and environment within which they are used, but also pose a hazard to those handling the reagents. The high throughput nature of forensic sampling means this is of utmost importance, as continued exposure to toxic reagents can result in subsequent health issues for the analysts working with them. Therefore, the toxicity of each NM should be considered in regards to how the developed biosensor will be applied, for example for field deployment or in a laboratory setting. By identifying the toxic characteristics, a more efficient and safer NM can be selected for the designated purpose [66]. The five main characteristics of a NM that can influence its toxicity are particle size, shape, surface charge, composition and coating [65]. In addition to considerations over the particle itself, thought must also be given to the exposure route. Exposure routes include but are not limited to, inhalation, ingestion, skin and injection. Therefore, when assessing the toxicity of NMs appropriate safety measures must be taken to ensure that these routes of exposure are controlled. Despite this, it is important to assess the toxicity and its effects on a case-by-case basis, as in some scenarios the toxicity of the nanomaterial may not pose an issue, whereas in certain scenarios where it may be detrimental to the target molecule or the person handling the reagent it may render the biosensor redundant.

3. Nanomaterial-based biosensors within forensic analysis

Within this review biosensors manufactured for the following forensic purposes have been considered: illicit drug screening, biological trace detection, environmental screening, and security and terrorism monitoring. These four branches of forensic science have been chosen due to the nature of their analysis. These fields could benefit greatly from the employment of sensitive, rapid and portable testing mechanisms for the detection of target analytes.

3.1. Anti-doping and illicit drug screening

Bulk drug analysis can be defined as the analytical investigation of bulk drug materials, the intermediates in their synthesis, drug formulations, impurities and degradation products [67]. The widespread use of illicit drugs across the world is an important issue faced by not only medical professionals but also within the forensic field [68]. Drug analysis is often needed within forensic analysis, for example for seized drug identification or within post-mortem samples. Due to the high throughput nature of drug screening, the need for simple and inexpensive, yet rapid and sensitive detection methods is evident. Currently employed methods validated by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), such as Liquid Chromatography/Mass Spectrometry, High-Resolution Mass Spectrometry or Spectroscopy, provide a high level of sensitivity and specificity, but often lack speed and require a laboratory setting for use, making analysis time consuming and relatively expensive [69,70]. The detection of many illicit drugs, such as cocaine, cannabis, amphetamines and opiates, via the use of novel biosensors is proving to be a viable alternative for the rapid and sensitive screening of drug samples, which can replace the timely conventional techniques [71–73]. The ability to multiplex biosensors to screen for different drugs at the same time also represents a major advantage, as unidentified samples can be tested for multiple drugs simultaneously. Notable biosensors for illicit drug detection have been outlined in Table 2.

Gold nanoparticles (AuNPs) have gained recent attention due to their strong optical absorption and unique electronic, chemical and biological properties. Of the various optical biosensing methods, colourimetric assays utilising AuNPs have been widely investigated as they are generally low cost, simple to conduct and the observation of a colour change is possible with the naked eye, limiting the need for further equipment [74]. Gold NPs display quasi-homogenous catalytic properties due to their easily controlled structural features, biocompatibility and high stability [75]. With a particle size of >5 nm, AuNPs are active catalysts and can participate in oxidation reactions. AuNPs have been noted to act as artificial enzymes that demonstrate peroxidase-like activity, which can catalyse substances to generate a coloured product [76]. This formation of a coloured product has been exploited in many optical biosensing systems, such as Soh et al., who have developed an aptamer-functionalised AuNP-based mechanism to detect cocaine in synthetic biological matrices [77]. Within this biosensor, the aptamer

Table 2
Optical biosensors designed for bulk drug and anti-doping analysis that have incorporated nanomaterials within their design.

Target Analyte	Nanomaterial	Mechanism	Sensor characteristics	Reference
Methamphetamine	Au@Ag core-shell nanoparticles	Colourimetric biosensor based on non-aggregation Au@Ag core-shell NPs. Au@Ag NP reporter probe, magnetic bead capture probe and aptamer raised against methamphetamine are used for detection. Aptamer hybridises to the reporter and capture probes, generating an Au@Ag-DNA magnetic bead sandwich. Surface plasmon resonance (SPR) intensity based detection, which is determined by the magnetic removal of the Au@Ag-DNA bead complex. SPR intensity is correlated with the concentration of methamphetamine.	Assay Time: 60 min LOD: 0.1 nM (14.9 ng L^{-1}) Linear range: 0.5 nM - 200 nM Selectivity: Selective for methamphetamine (insignificant response from KET, NK, MOR, COC, CAT, MCAT, BZP, and MDA) Storage:	[92]
Cocaine	Silica nanoparticles and gold nanoparticles	Fluorescent aptasensor based on the hairpin structure of a complementary aptamer strand and the target-induced release of a cocaine binding aptamer from a complementary stand and silica NPs (SNPs) coated with streptavidin and gold NPs. In presence of cocaine, aptamer binds with target, and is released from complementary strand. Fluorophore (FAM) on the complementary strand comes into close proximity with the surface of SNPs due to the formation of the hairpin structure of the complementary strand, resulting in a strong fluorescence emission.	Assay Time: LOD: 0.209 nM Linear range: 0.5–20 nM Selectivity: Selective for cocaine (insignificant response from diazepam, propranolol and chloramphenicol) Storage:	[93]
Codeine	Gold nanoparticles	Citrate-capped AuNPs form a drug-nano complex, which causes absorption spectra changes in the presence of codeine sulphate. Codeine sulphate molecules possess many binding sites that allow for strong coordination to AuNPs via the ligand exchange with weak surface-bound citrate ions and cross-linked AuNPs. The linker-based aggregation is responsible for the rapid detection.	Assay Time: <60 s LOD: 0.9 μM Linear range: 1 mM–20 mM Selectivity: Selective for codeine sulphate (no response from morphine, phenylephrine, phenobarbital, diazepam, lorazepam or clonazepam) Storage: stable for several months	[94]
γ-Hydroxybutyric acid (GHB)	Gold nanoparticles	Biosensor that utilises the fact that GHB can be detected via a specific enzyme-substrate interaction that generates NADH as a by-product. Formed NADH facilitates the reduction of gold (III) species to generate AuNPs that are analysed by an absorbance spectrometer or the naked eye.	Assay Time: 75 min LOD: 3.51 mM Linear range: 4–16 mM Selectivity: Selective for GHB vs GBL (γ-butyrolactone) Storage:	[95]

raised against cocaine is initially adsorbed onto the surface of the AuNPs via Au-nucleotide affinity. The Au-nucleoside affinity is controlled by non-covalent forces including electrostatic interactions, hydrophobic forces, and specific bonding between the chemical groups of the DNA bases and the gold surface [78]. Upon interaction with cocaine in the sample, the cocaine molecules cause desorption of the aptamer strand from the AuNP surface. If cocaine is not present within the sample matrix the aptamers will remain adsorbed to the AuNP surface. Upon the addition of hydrogen tetrachloroaurate (III) ($HAuCl_4$) and hydroxylamine (NH_2OH), which are needed for AuNP growth, the AuNPs will grow in either a spherical or branched manner. If cocaine is present within the solution fewer aptamers will remain on the surface of the AuNP, resulting in the growth of spherical AuNPs, which are red in colour. When cocaine is not present within the solution the AuNPs will retain high aptamer coverage and will grow into branched NPs resulting in a blue-coloured solution (Fig. 1). The developed aptasensor produced a visible colourimetric response and was able to detect cocaine with a LOD of 1 nM.

This biosensor mechanism is an example of a simplistic optical output signal that can indicate a positive target interaction. The combination of a low LOD and distinctive optical signal generation between target presence and absence makes the incorporation of AuNPs desirable in biosensors for rapid illicit drug screening. A further benefit of AuNPs is that various synthetic routes produce AuNPs of differing size or shape, which influence their properties. The different available morphologies of AuNPs result in alternative optical, catalytic and electronic behaviour, meaning they can be tuned to give a desirable mechanism and output method depending on their use. This ensures that they are well suited for use within forensic biosensors where the optical output method is important for its easy application and detection of target molecules [79]. However, the drawback of incorporating AuNPs into optical biosensors is that they can be costly to manufacture, which could prevent them from being used for analysis where large quantities of biosensor reagents are needed (e.g. pitch-side doping testing, crime scene investigation or environmental screening). Furthermore, it has been noted by Jans and Huo that the use of AuNPs for enabling metal-enhanced fluorescent emissions within biosensors is still an underdeveloped field that requires additional research into their properties before their widespread commercial deployment [80].

Another mechanism being utilised within optical biosensing platforms is controlled-release fluorescence. Lozano-Torres et al., have reported a biosensor that is capable of detecting 3,4-methylenedioxymethamphetamine (MDMA) in water by utilising psuedorotaxane capped mesoporous silica nanoparticles that function via a molecular gate mechanism (Fig. 2) [81]. Mesoporous silica NPs are widely used within medical biosensors for the delivery of drugs. They pose the advantages of uniform and tuneable pore size, simple surface functionalisation, and a gating mechanism that allows for controlled release of internally held molecules [82]. Detection of the target analyte is achieved as the pores within the nanoparticles are initially blocked by psuedorotaxane molecules (formed between a grafted naphthalene derivative and a $CBPQT^{4+}$ macrocycle), preventing the release of the fluorescein molecules that are held within the cavities. Upon interaction of psuedorotaxane with MDMA, the psuedorotaxane groups are displaced from the surface of the NPs due to the formation of MDMA-$CBPQT^{4+}$ donor-acceptor supramolecular complexes. Aqueous suspensions of the biosensor show a negligible fluorescent emission as the fluorescein is shielded within the NPs. However, in the presence of MDMA, notable fluorescent emission is seen at 520 nm, indicating a positive target interaction as the fluorescein is released from the NPs [83]. The resulting biosensor was able to detect MDMA with a LOD of 4.9 μg/mL in water.

Drugs that are prohibited by the World Anti-Doping Agency (WADA) are of interest to forensic chemists when considering Anti-Doping Rule Violations (ADRVs). An ADRV is defined as when an athlete or a member of their supporting team commits a doping offence and as a result, there

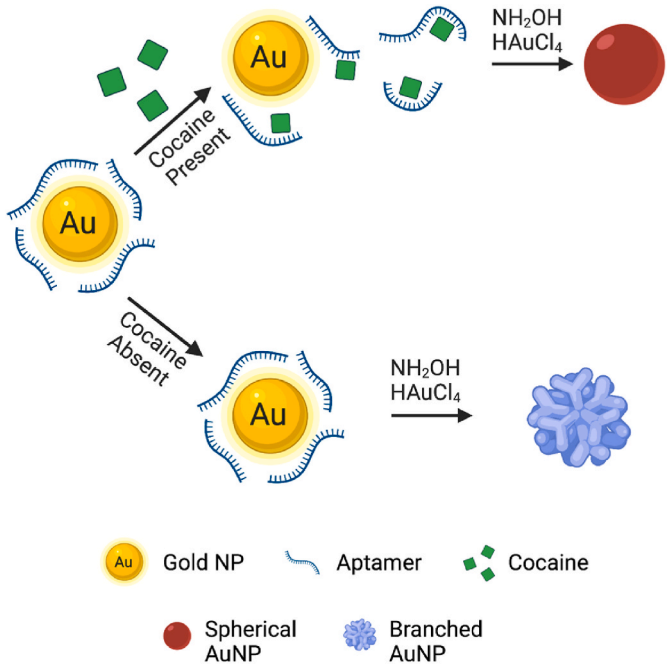

Fig. 1. Colourimetric AuNP biosensor for the detection of cocaine molecules designed by Soh et al. In the presence of cocaine, the interaction between aptamer and target causes desorption of the aptamer from the AuNP surface. If cocaine is not present within the sample, the aptamers will remain adsorbed to the AuNP surface. Upon the addition of NH_2OH and $HAuCl_4$, varying morphologies of the NPs are produced, forming different colours of grown NPs. AuNPs that have low amounts of aptamer on their surface are spherical and red in colour, indicating cocaine molecules are present. AuNPs that have high amounts of aptamer on their surface, indicating no cocaine molecules present, grow into a branched morphology and form a blue colour. Figure adapted with permission from Ref. [77]. Copyright 2015 American Chemical Society. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

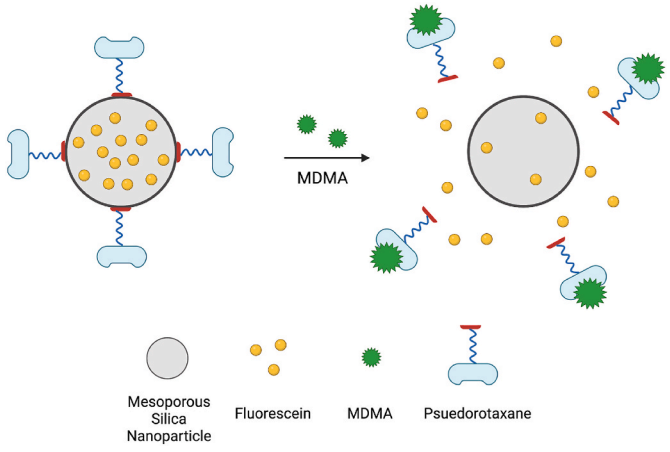

Fig. 2. Controlled-release fluorescent biosensor designed for the detection of MDMA developed by Lozano-Torres et al. Mesoporous silica nanoparticles loaded with fluorescein are initially capped with a psuedorotaxane. In the presence of MDMA, the psuedorotaxane is displaced due to preferential binding with MDMA, which allows the fluorescein to be released. Figure adapted with permission from Ref. [81].

are consequences or sanctions to that person regarding their participation in future competition [84]. The introduction of biosensing and hence the incorporation of nanotechnology to anti-doping detection is significant as biosensors may be an invaluable improvement to current analysis by offering alternative confirmatory testing alongside conventional anti-doping detection methods [85]. Such an introduction would bring the advantage of portability meaning that pitch-side testing could be carried out rapidly. The ability to perform a rapid pitch-side test would remove the need for all samples to be sent to a laboratory meaning only samples that showed a positive result during biosensor-based screening would need to be analysed for further quantification [86].

Platelet-Derived Growth Factor (PDGF) is a dimeric glycoprotein consisting of either two A (-AA) or two B (-BB) polypeptide chains, or a mixture of the two (-AB) and is a banned growth hormone by WADA. Li et al. designed a metal NP-based fluorescent biosensor that is capable of detecting PDGF-BB via the use of fluorophore-functionalised aptamers containing Black-Hole Quencher 2 (BHQ-2) [87]. For this biosensor, streptavidin (SA) was initially conjugated to AgNPs. The functionalisation of SA to the AgNPs serves to aid in the conjugation of the aptamers. The aptamers raised against PDGF-BB not only carry the TAMRA fluorophore but also contain terminal biotin. The affinity between biotin and SA has been exploited in molecular biology and bionanotechnology due to its stability, making it one of the strongest non-covalent interactions known in nature ($K_d = 4 \times 10^{-14}$ M) [88]. Additionally, its resistance to extreme pH, temperature, organic solvents, denaturants and proteolytic enzymes makes it an ideal method for chemically conjugating aptamers to NPs [89]. Aptamers raised against PDGF-BB carrying TAMRA (fluorophore), and a quencher carrying strand containing biotin, were hybridised in duplex. These aptamers were then bound with the SA-functionalised AgNPs to form a FRET biosensor (Fig. 3) [87]. FRET is the occurrence of non-radiative energy transfer between two fluorescent molecules, which occurs as a result of an electrostatic dipole-dipole interaction [90]. An excited donor fluorophore emission is quenched by alternative transmission to a nearby acceptor molecule. The distance between donor and acceptor is tightly controlled, with a distance of >10 nm alleviating quenching effects and permitting signal output [91]. The FRET proximity between the fluorophore and quencher results in decreased fluorescence when no PDGF-BB is bound. Upon interaction of the fluorophore-functionalised aptamers with native PDGF-BB, the

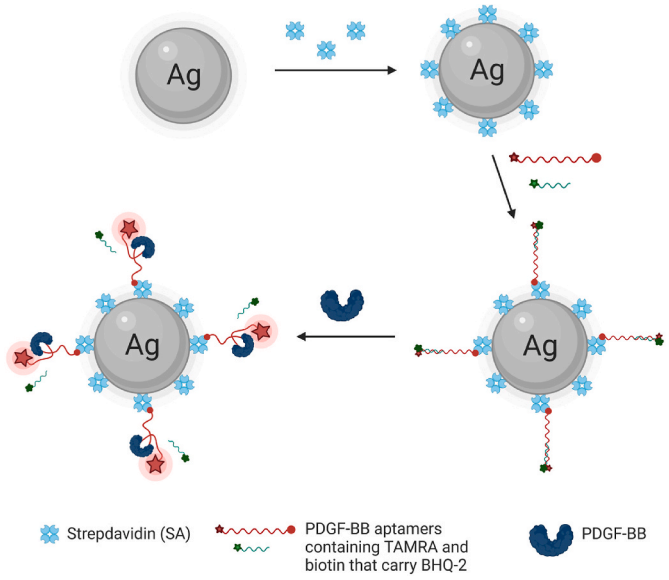

Fig. 3. AgNP-based biosensor for the detection of PDGF-BB developed by Li et al. (a) AgNPs are functionalised with SA. (b) The SA-AgNPs are then incubated with quencher-labelled Biotin-Aptamers containing TAMRA fluorophore. The binding brings the fluorophore and the quencher into close proximity (FRET) which initially quenches fluorescence. (c) When the biosensor interacts with PDGF-BB, the quencher strand is displaced and hence fluorescent emission occurs. Figure adapted with permission from Ref. [87]. Copyright 2013 American Chemical Society.

BHQ-2 carrying strands of the duplex are displaced, meaning the FRET distance is disrupted; the fluorescence is therefore restored making the positive target interaction detectable. The resulting LOD of the biosensor was 0.8 ng/mL for PDGF-BB, which represents a 16-fold increase in sensitivity vs the bare FRET biosensor without the inclusion of NPs.

Silver has been noted to exhibit advantageous catalytic properties over other metallic NMs, such as gold, making them well suited for incorporation into biosensors. As demonstrated above, the addition of AgNPs to a biosensor allows for an increase in sensitivity. This can be attributed to the large surface area of the AgNPs, which allows for an increased number of biological recognition moieties to be immobilised on the surface. This also serves to enhance the signal output, making a positive target interaction easier to record [96]. Further advantages include widely tuneable plasmonic properties, meaning that the manipulation of the size and shape of the NP allows for variation in their localised surface plasmon resonance leading to variation in the observable colour [97]. Furthermore, AgNPs have a well-mastered synthesis and fabrication methodology, making them relatively simple to incorporate within a biosensor design. Despite this, AgNPs remain far less utilised within the field of biosensor development [98]. The reason for this is that AgNPs demonstrate lower chemical stability compared to other forms of metallic NPs, rendering them less reliable for use in biosensors. The lower chemical stability is problematic for forensic biosensors, where reliability and reproducibility of testing are paramount, as this can compromise efficient target analyte detection.

3.2. Biological trace detection

Biological traces can be defined as a range of human bodily fluids and tissues that can be transferred through physical contact. These include biological fluids, such as blood, semen and saliva, as well as human tissues, such as hair, teeth, nails and bone. The detection of biological traces is fundamental to forensic investigations as they may provide a source of genetic material, as well as investigative information. Whilst biosensors for the detection of traces such as DNA and fingerprints are reported in the literature, many of them do not have an optical transduction mechanism, instead relying on electrochemical platforms. The majority of reported optical biosensors that incorporate nanomaterials into their design are targeted toward biological fluid detection. Therefore, this is the application that has been chosen for inclusion in this review.

Biological fluids are frequently encountered at crime scenes, with semen, blood and saliva forming the most common biological evidence recovered. Currently employed presumptive tests for these fluids often lack specificity, the ability to be multiplexed into a single assay and can be sample destructive. Biosensors may be able to facilitate large-scale high-throughput, sensitive in situ screening to detect stains that are of high evidential value or a source of genetic material that can lead to the identification of a suspect or victim. The examples given below have been chosen due to their ease of signal detection, which is required within forensic casework, and also their suitability for portable, rapid, sensitive testing of biological traces. Optical biosensors for biological trace detection and identification have been outlined in Table 3.

Recent research efforts have utilised Qdots to develop biosensors for the detection of bodily fluids. The successful application of antibody-functionalised Qdot nanoparticles for use within a 'turn-on' fluorescent displacement biosensors for the detection of prostate-specific antigen (PSA) within semen has been demonstrated [99]. This biosensor utilises antibodies that are capable of specific binding with PSA as the biological sensing moiety. It is thought that the interchangeable nature of Qdots and fluid antibodies highlighted within this work could be readily applied for the detection of different bodily fluids, such as blood and saliva. The biosensor design presented within this work uses Qdot NPs conjugated to PSA-specific antibodies to produce a fluorescent biological recognition complex. This complex was then provisionally quenched via the binding of a quencher-labelled peptide analogue (H–DVCAQV–NH_2) to PSA. This analogue was brought within the 10 nm distance needed for the absorbance of the Qdot emission via FRET to occur, resulting in a 'turn-off' of biosensor emission signals. When the biosensor complex comes into contact with native PSA within seminal fluid, the peptide analogue is displaced by the higher affinity native protein, which in turn restores the fluorescence (Fig. 4). Within this biosensor, the excellent optical properties of Qdots allow for a simple transduction output mechanism. The narrower emission band and wider absorption bands of Qdots (when compared with traditional organic fluorophores) allow for greater control of optical features, such as their

Table 3
Optical Biosensors for the detection of biological fluids that have incorporated nanomaterials within their design.

Target Analyte	Nanomaterial	Mechanism	Sensor Characteristics	Reference
Human Semen	Zirconium metal-organic frameworks and Gold nanoparticles	Colourimetric biosensor that couples zirconium metal−organic frameworks (Zr-MOFs) with ssDNA-decorated AuNPs (ssDNA-AuNPs). Coordination interactions between zirconium clusters and DNA phosphate backbone, in combination with π−π stacking and H-bonding, allows Zr-MOFs to absorb and precipitate AuNPs with the assistance of ssDNA. If human semen is present, the colour of the supernatant will change.	Assay Time: 2 h LOD: Linear range: Selectivity: Identification accuracy = 90% Storage:	[114]
Prostate Specific Antigen (PSA)	Polyamine nanoparticles with magnetic iron oxide cores	Biosensor that uses magnetic beads functionalised with *anti*-PSA antibodies and gold nanoparticle probes. Consists of a DNA oligonucleotide sequence and a specific antibody that binds to the target that is initially captured by the antibody on the magnetic beads. After magnetic separation of bound complex, PSA concentration is determined by measurement of the oligonucleotide levels, which are directly related to concentration of PSA.	Assay Time: LOD: 30 aM Linear range: Selectivity: Storage:	[115]
Glycophorin A	CdSe/ZnS Quantum Dots	An *anti*-glycophorin A antibody (Ab) is conjugated to fluorescent semiconductor Qdots via sulfhydryl chemistry. When liquid blood samples were incubated with Ab-Qdots, fluorescence was quenched/altered in a concentration-dependant manner. Full DNA profiles were obtained from blood samples that were treated with the Ab-Qdot showing that the biosensor does not interfere with profiling.	Assay Time: 30 min LOD: Linear range: Selectivity: No detection seen with semen or saliva Storage: 4 °C in the dark	[116]
Acid Phosphatase	$CuInS_2$ Quantum Dots	Fluorescent 'turn off-on' biosensor based on aggregation-caused quenching and enzymolysis. When treated with sodium hexametaphosphate $(NaPO_3)_6$, $CuInS_2$ Qdots aggregate via electrostatic interactions. This causes natural fluorescence of Qdots to be reduced due to aggregation caused quenching. When AP is present, $(NaPO_3)_6$ digests AP into phosphate fragments, which disturbs the aggregation of Qdots. This restores the fluorescence of the Qdots, indicating a positive detection.	Assay Time: 2 min LOD: 9.02 nU mL^{-1} Linear range: 75–1500 nU mL^{-1} Selectivity: little interference from commonly existing substances Storage:	[117]

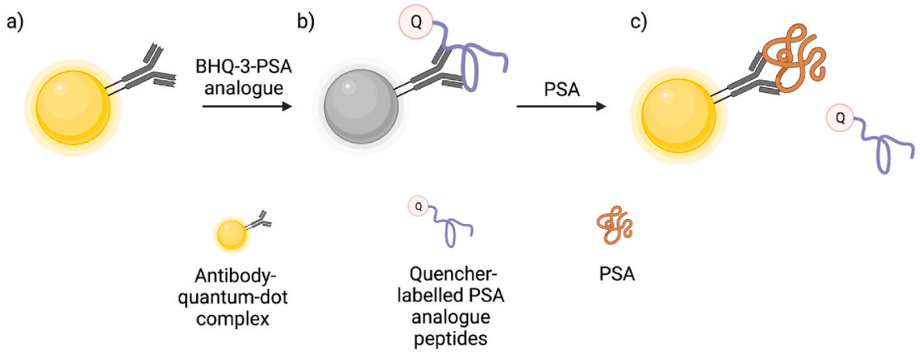

Fig. 4. The mechanism of a PSA biosensor to detect seminal fluid was designed by Frascione et al. (a) Antibody-quantum-dot complexes are produced via amine-thiol crosslinking. (b) This complex is then incubated with a quencher-labelled PSA analogue peptide. The binding brings the Qdot and the quencher into close proximity which quenches emission through FRET. (c) When in contact with native seminal PSA, the higher affinity binding of native PSA means that the quencher-labelled analogue peptide is displaced, and fluorescent emission is restored. Figure adapted with permission from Ref. [99].

emission colour and brightness. With a higher quantum efficiency and higher molar absorption coefficient than organic dyes, Qdots are seen as superior for inclusion in biosensors. These features make optical detection more sensitive, reliable and allow for multiplex analysis [100].

Despite the success of Qdot-based biosensors in providing sensitive and rapid signals for bodily fluid identification, there are health and safety concerns that may prevent their use within forensic analysis. In vitro toxicity caused by Qdots includes reduction of cell viability, genetic material damage and disordered immune cell reactions and the toxic effects displayed are dependent on composition, dose, size, surface chemistry and structure [101]. This means that Qdots can be toxic to the user when appropriate safety measures are not followed, such as suitable personal protective equipment (PPE). Furthermore, Qdots are reported to harm the respiratory system which could be extremely damaging over long-term inhalation of the biosensor reagents by crime scene personnel dispersing them [102]. Despite this, biosensor reagents containing Qdots remain a viable choice for use in an enclosed environment, such as a fume hood in a laboratory, although this limits the use of such biosensors at crime scenes. A further drawback is that the surface chemistry influences the Qdot's propensity to aggregate, particularly in biological solutions. This may present an issue if the biosensing units are applied to a biological sample as there is a possibility that Qdots may aggregate and damage any genetic material present that may be later required for DNA analysis. DNA evidence could potentially be compromised by the photocatalytic generation of reactive oxygen species (ROS) from the Qdots that can damage cellular proteins, lipids and DNA, causing cytotoxicity [103,104]. Qdots may be more suited to analysis where genetic material is not a vital constituent within the sample, such as bulk drug analysis or explosives detection.

Novel work presented by Li et al. demonstrated how bacterial targets can be detected that are unique to the microbiome of human fluids for the highly specific detection and identification of saliva [105]. This optical biosensor uses bovine serum albumin (BSA) stabilized Silicon Carbide (SiC) nanoparticles (SiC@BSA NPs) conjugated with antibacterial peptide GH12 to detect the oral bacteria *Streptococcus salivarius* (Fig. 5). Whereas previously outlined biosensors utilise sensing moieties such as antibodies and aptamers, this biosensor utilises the antibacterial peptide - GH12. Conjugation of the GH12 peptide to the SiC@BSA NPs' surface is achieved via the interaction between the terminal amino group of GH12 and carboxyl groups of the BSA NPs via EDC/NHS coupling. *S. salivarius* is known to be a health-associated bacteria that is most commonly found on the dorsum of the tongue and the pharyngeal mucosa [106]. It is one of the first colonizers of the human oral cavity and gut from a few hours after birth and remains within the oral cavity as a predominant commensal inhabitant [107]. The bacterial strain has demonstrated its suitability as a salivary biomarker for diseases [108] and is now being utilised as a biomarker for the detection of human saliva itself for forensic purposes. The principles of the biosensor mechanism can be seen in Fig. 5. When the prepared biosensor is in the presence of *S. salivarius,* GH12 couples with the bacterial membrane. Despite research being conducted into this interaction of peptide and

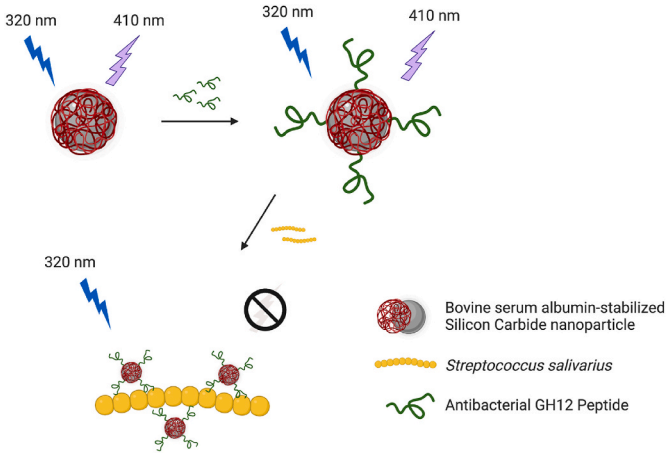

Fig. 5. SiC@BSA NP-based biosensor to detect the oral bacteria *S. salivarius* designed by Li et al. The biosensor design uses bovine serum albumin-stabilized SiC nanoparticles (SiC@BSA NPs) conjugated with antibacterial peptide GH12. After the interaction of GH12 with the SiC@BSA NPs, a decrease in fluorescence intensity at 410 nm is recorded. Figure adapted with permission from Ref. [105].

membrane [107,109,110], the exact binding mechanism remains unknown. Once successful target interaction has taken place, the fluorescence of the SiC@BSA NPs is quenched as a result of electron transfer from the SiC@BSA NPs to *S. salivarius* via FRET. Therefore, fluorescence intensities can inversely be measured as a function of the *S. salivarius* concentration [105]. Results showed that *S. salivarius* within human saliva could be detected with a LOD of 25 cfu mL^{-1} and the saliva sample could be successfully differentiated from semen, blood and urine using this biosensor. Additionally, the use of antimicrobial peptides as the sensing element has clear advantages over traditionally used antibodies, including small size, good stability towards bacterial targets and chemical stability.

SiC NPs have been widely used within biosensor designs for medical analysis due to their low cost and good commercial availability, which has resulted in them being subsequently utilised within forensic biosensors. SiC NPs display mechanical and chemical stability due to the short lengths of the covalent bonds between the Si and C atoms [111]. Due to these properties, alongside high thermal conductivity, SiC is considered a suitable NM for incorporation within biosensors where high temperatures or variable environments are encountered [112]. Additionally, the chemical inertness of SiC has resulted in resistance to corrosion within certain testing matrices, such as bodily fluids, making them an ideal addition to optical biosensors for bodily fluid detection at crime scenes. However, cytotoxicity caused by SiC NPs is a concern, but it is thought to be cell-dependant [113]. Toxicity displayed by SiC NPs is of a varying degree and is dependent on the form of SiC that is used, so

prior to incorporation into the biosensor design, the target organism and SiC form must be considered to control toxicity where possible.

3.3. Environmental, security & terrorism screening

Environmental monitoring has long been of forensic interest due to its role in public and environmental health. The monitoring of contaminants in the air, water and soil is vital to managing any possible risks that may affect human health and ecosystems. With modern technology and industrial practices, there is an increasing number of damaging pollutants entering the global environment [118]. This in turn requires cost-effective and efficient analytical techniques that can be used to detect such pollutants. The ability of a biosensor to function within complex matrices is of importance, as it means that detection can occur without the need for extensive sample preparation, such as solid phase extraction, which is usually required due to the nature of the sample (such as water sources, air samples or soil samples) [119]. Similar to environmental monitoring of contaminants, screening for molecules associated with terrorist attacks is of great importance within forensic science. The detection of explosives and biological warfare agents is becoming increasingly studied as it contributes to public safety. Bioagents and explosives pose immediate health risks, therefore their detection must be fast and accurate. The ability of a biosensor to produce a signal output in real-time is necessary within environmental, security and terrorism screening where public health and safety rely on rapid intelligence. Other reported biosensors for environmental, security and terrorism screening identification have been outlined in Table 4.

The detection and quantification of inorganic arsenic within various matrices are of utmost importance to the environment and human health due to the hypertoxicity and carcinogenicity of arsenic. Two common forms of inorganic arsenic that can accumulate within the body via the food chain are pentavalent arsenate (As^{5+}) and trivalent arsenite (As^{3+}) [120]. Enzymatic biosensors have been developed for the rapid detection of arsenic, which relies on the principle of arsenic being a heavy metal ion, so it can bind certain natural enzymes which, as a result, can influence their catalytic activity via inhibition. Nanomaterials can be incorporated into enzymatic biosensors to act as signal indicators, giving them an optical output. Upon the addition of enzymes to the solution of interest, the corresponding substrate for the enzyme is decomposed, resulting in a change in signal output. When using an enzymatic biosensor to detect arsenic, if arsenic is present, it will inhibit the catalytic activity of the enzyme, which can affect signal generation. A fluorescent nanoprobe for the detection of arsenic was recently developed by Wen et al. that uses carboxylated Cadmium Selenide Zinc Sulphide Quantum Dots (CdSe/ZnS Q-dots), Terbium complex III (Tb(III)) and guanosine monophosphate (GMP) to detect arsenic in environmental water samples [121]. The designed biosensor has two separate emissions - red and green - from the Qdots and the Tb(III)-GMP

Table 4
Optical biosensors designed for the purpose of environmental, security or terrorism screening that have incorporated nanomaterials within their design.

Target Analyte	Nanomaterial	Mechanism	Sensor Characteristics	Reference
Organophosphorus (OP) Neurotoxins	Gold nanoparticles	AuNP fluorescence biosensor that utilises NP-modified fluorescence of an inhibitor of organophosphorus hydrolase (OPH) enzyme to generate a signal for OP detection. AuNP is covalently bound to the OPH enzyme and is attached to a fluorescent analogue that is a weak competitive inhibitor of OPH due to its similar chemical structure to OP. Upon interaction with native OP, analogue is displaced, causing a change in fluorescence of the analogue due to its altered distance from AuNP.	Assay Time: LOD: 20 μM Linear range: 20–240 μM Selectivity: Storage:	[132]
Salmonella paratyphi A	Carbon nanotube	Biosensor based on non-covalent self-assembly of SWNTs and DNAzyme-labelled aptamer probes. The DNAzyme sequences acts as a label to amplify detection signals, whilst the aptamer shows high affinity binding to the bacteria. Aptamer probe forms a stable complex with the SWNT. Upon addition of hemin and target analyte, the probe is displaced from SWNT, and forms a complex with the target. This causes a change in measurable fluorescence.	Assay Time: 15 h LOD: 1.0×10^3 CFU mL^{-1} Linear range: 1.0×10^3–1.0×10^7 CFU/mL Selectivity: no cross-reaction with other *Salmonella* serovars and pathogens Storage:	[133, 134]
2,4,6-trinitrotoluene (TNT)	Semiconductor Quantum dots	FRET-based biosensor consisting of anti-TNT specific antibody fragments attached to hydrophilic Qdots via metal-affinity coordination. Initially, a dye-labelled TNT analogue is prebound in the antibody binding site, which quenches the Qdots photoluminescence via proximity-induced FRET. When biosensor comes into contact with TNT, the dye-labelled analogue is displaced from the anti-TNT specific antibody fragments, which disrupts FRET, recovering the Qdots photoluminescence.	Assay Time: LOD: 20 ng/mL Linear range: 20 ng/mL - 10 μg/mL Selectivity: moderate cross reactivity seen with Tetryl and 2-A,4,6 DNT, negligible with 2,6-DNT Storage:	[135]
Aspergillus amstelodami	Quantum dots	Turn-on biosensors formed via conjugation of IgG antibodies to Qdots and incubation with quencher-labelled analogues to *Aspergillus amstelodami.* The FRET-based biosensor allows for Qdot energy to be transferred to the quencher species, which results in reduced fluorescent emission from the Qdot donor. Upon interaction with the native target analyte, the quencher-labelled analogues are displaced by the higher affinity target analytes, which creates a detectable fluorescence signal increase from the Qdot donor.	Assay Time: 10 min LOD: 1.0×10^3 spores/mL Linear range: 1.0×10^3–1.0×10^5 spores/mL Selectivity: no cross reactivity seen with *E. coli* O157:H7 Storage:	[136]
Calcium dipicolinate (CaDPA) (biomarker for *B. anthracis* spores)	Silica nanoparticles	Silica NPs are initially doped with fluorescein isothiocyanate (FITC) and an organic complex, ethylenediaminetetraacetic acid dianhydride (EDTAD), is then covalently coordinated to the surface of the NPs. The sensing moiety, Europium (Eu^{III} complex), is conjugated to the surface of the organic-dye-doped silica NPs. Detection of CaDPA causes water molecules to be excluded from the Eu^{III} complex, which minimises the non-radiative quenching of the Eu^{III} complex. This is detected via an increase in luminescence from the Eu^{3+} due to water sensitivity. The addition of CaDPA results in an improvement in the emission intensity from the Eu^{3+} complex, while emissions from FITC remains constant.	Assay Time: 2 min LOD: 0.2 nM Linear range: 0.6–600 nM Selectivity: shows selectivity for CaDPA over aqueous ligands Storage:	[137]

coordination polymer, respectively. After acid phosphatase enzymes (AP) are added to the sensing unit, hydrolysis of the GMP component takes place, with the destruction of the Tb (III)-GMP coordination polymer structure. This results in the quenching of the green emissions from the coordination polymer, with the red emissions from the Qdot remaining. When the target analyte, arsenate, is present within the testing matrix the enzyme inhibition of AP by the arsenate prevents the decomposition of the coordination polymer. Therefore, arsenate can be detected by the presence of the dual emission of the biosensing unit, with a lack of arsenate indicated by the turn-off of the green emissions from the Tb(III)-GMP coordination polymer (Fig. 6).

The biosensor mechanism of enzyme inhibition has been used within the medical field and is becoming more readily employed within biological analysis. Despite the advantages of sensitive detection and efficient optical signal output via the coloured dual emissions, there are some drawbacks to this detection mechanism. The use of AP, a natural enzyme, may pose a challenge due to environmental factors that may be hard to control. For example, if the enzyme is exposed to extreme heat or a pH change, this may lead to denaturation of the enzyme, which in turn will not decompose the coordination polymer leading to false negatives, which cannot be tolerated within a forensic setting. Additionally, natural enzymes can be relatively expensive and their use within the mechanism poses additional difficulty [120]. Finally, it is possible that any metal ions that are present within the testing matrix, such as Lead (Pb(II)) and Mercury (Hg(II)), could inhibit AP, preventing interaction with arsenate and hence lowering sensitivity. Therefore, whilst this mechanism is efficient for testing that is carried out in a controlled laboratory environment, the variable nature of enzymatic-based biosensors may render them unsuitable for certain field applications.

Detecting chemical explosives, such as Trinitrotoluene (TNT), is paramount for military and civilian safety [122]. A range of cost-efficient and portable testing mechanisms have been developed in recent years for the detection of explosives, however, biosensors are gaining attention as they can offer trace detection of explosives, bringing an enhanced selectivity and sensitivity. Zhang et al. reported a graphene-oxide-based optical biosensor that utilises functionalised peptides to detect TNT (Fig. 7) [123]. Within this biosensor, graphene oxide (GO) was cross-linked with TNT-specific peptides (WHWQRPLMPVSI) via EDC/NHS coupling through dehydration condensation reactions between carboxyl groups on the GO sheets and amino groups on the peptides, hence forming peptide-functionalised GO. In the presence of TNT, binding of the peptides to the target

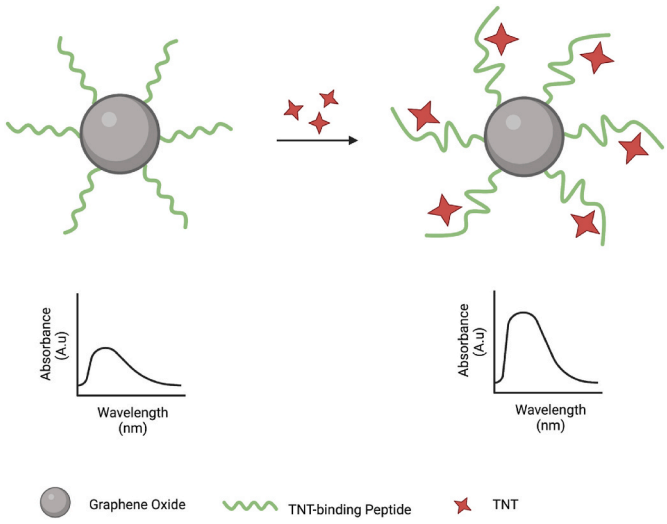

Fig. 7. Graphene oxide-based optical biosensor for the detection of the explosive TNT, developed by Zhang et al. Graphene oxide is cross-linked with TNT-binding peptides that demonstrate highly specific binding to TNT molecules. Upon binding of TNT to the biosensing unit, a change in absorption is seen. Figure adapted with permission from Ref. [123].

molecule can induce an increase in the absorption magnitude in the optical spectrum of the GO at ~238 nm, which has been demonstrated to be dose-dependent. This increase in adsorption is thought to be due to the donor-acceptor interactions and π-π interactions between the peptide chains and TNT molecules. Due to the structural similarity between TNT and 2,6-dinitrotoluene (DNT), Zhang et al. also reported that this biosensor is highly selective for TNT with a LOD of $\sim 4.40 \times 10^{-9}$ mM, showing distinctive absorption differences from DNT molecules.

GO sheets are the product of chemical exfoliation of graphite and possess many polar oxygen-containing groups, which allow them to be easily modified by various species such as peptides, nucleic acids and proteins (Table 1) [123,124]. The use of GO within optical biosensors brings the advantages of optical detection whilst being simple to synthesise and demonstrating a quick response time [123]. Their high surface area allows for good biocompatibility with sensing moieties, such as peptides, DNA, enzymes and antibodies [125] which can increase the sensitivity of the probe. Despite the advantages of

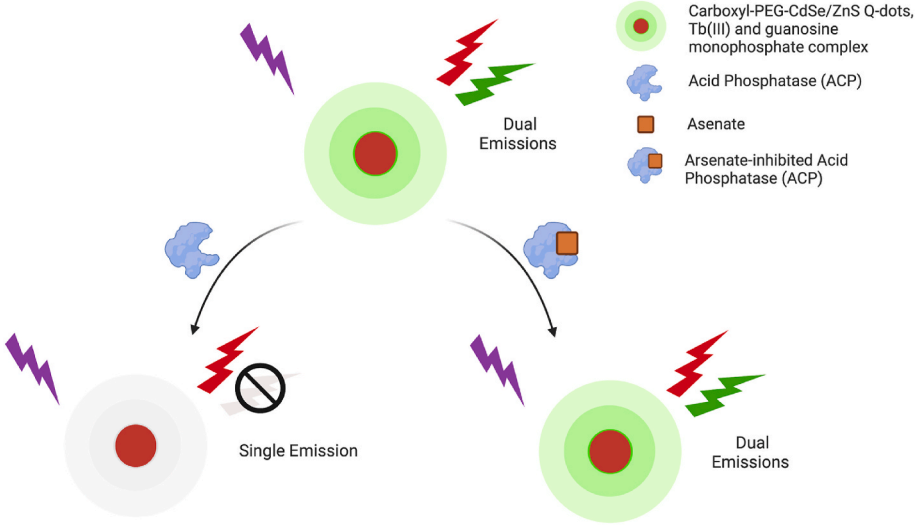

Fig. 6. Optical biosensor designed for the sensitive detection of arsenate within water samples developed by Wen et al. The fluorescent biosensor incorporates CdSe/ZnS Q-dots, Tb(III) and guanosine monophosphate to detect arsenate via its inhibition of natural acid phosphatase enzymes. Figure adapted with permission from Ref. [121].

incorporating GO within a biosensor it is crucial to consider the variables that may affect the selectivity and sensitivity of the biosensor. These variables include the orientation of the sheets, the number of layers, the functional groups and oxidation states of graphene, which can all also affect the binding between transducer and sensing moiety. Additionally, non-specific adsorption sites, such as unused oxygen functional groups on the GO, must be considered, which can be blocked to prevent any non-specific binding via the use of blocking agents. By controlling all variables associated with the use of graphene-based nanomaterials, future work can use this type of optical biosensor as a basis for designing biosensors capable of detecting other forensically relevant targets.

A recent study by Wang presented a near-infrared (NIR) optical biosensor that is also capable of detecting TNT using peptide functionalised single-walled carbon nanotubes (SWNTs) (Table 1) (Fig. 8) [126]. Within this biosensor design, a TNT binding peptide (ARGYSSFIYWFFDFC) which was initially derived from an anti-TNT monoclonal antibody, is anchored to the side of a SWNT via π-π stacking interactions [127,128]. A positive target interaction of the TNT binding peptide with TNT molecules is signalled via a change in the absorbance of the SWNTs in the NIR spectra. When a TNT molecule binds to the TNT recognition peptide via π-electron-mediated effects (of the aromatic amino rings within TNT) and hydrogen bonding, the NIR absorption intensity at 1930 nm increases in a dose-dependent manner over the range 0.01–10 ppm TNT. The LOD of this biosensor was reported as 0.01 ppm [126]. Additionally, it was demonstrated that the TNT binding peptide showed high specific binding to TNT molecules as opposed to TNT analogues.

SWNTs demonstrate a range of properties depending on their atomic regulation, diameter and length of the tubes making them a versatile NM [129]. They are fluorescent in the NIR, (900–1600 nm) due to their electronic band-gap between valence and conduction bands [130]. The chirality of the SWNT gives rise to different electronic properties. This in turn can result in different optical features. Their fluorescence is exploited within optical biosensors and is used to aid in transduction within this TNT-detecting biosensor. CNTs possess a large specific surface area, meaning a greater number of recognition moieties can be attached to the surface, leading to a higher sensitivity of detection of the biosensor. However, the fabrication of CNTs is difficult to control, meaning the resultant biosensor may vary in its properties. A further drawback is that CNTs are not as cost-efficient or impurity-free as desired [131], meaning that their use within forensic analysis may be limited. Before biosensor implementation into the analytical workflow, further research must address concerns linked to the stability of biosensors as well as the reliability of the biosensing units, to avoid raising a false positive.

4. Discussion and conclusion

From the biosensor mechanisms outlined within this review, it is evident that the incorporation of NMs within biosensors is a rapidly evolving field as demonstrated by a large increase in published articles relating to the field between 2010 and 2020 [138]. This article provides an overview of promising forensic biosensors that have demonstrated successful target identification with sensitivity, stability or ease of detection being enhanced via the incorporation of NMs. These specific biosensors were chosen for inclusion within this review for several reasons such as their low LOD, high specificity and compatibility within a forensic setting. Despite this evolution of research, it is evident that the employment of such biosensors within the forensic workflow is still behind other fields such as medicine, with limited optical NM-based biosensors being used within the field and casework.

It is shown that a FRET-based biosensor is the most prominent mechanism for a biosensor for forensic analysis due to its popularity within current literature and the successful results such biosensors have produced in regards to specificity for the target analyte and reliable optical output. However, in order to incorporate NMs within FRET-based biosensors, the FRET distance between the donor and acceptor must be tightly regulated and controlled. This is to ensure that optimal intermolecular interactions are present to render the acceptor and donor a FRET couple, and also to allow them to become dissociated in the presence of the target analyte [139]. Therefore, the design and manufacture of these biosensors must be rigorously scrutinised and optimised before their use with forensic samples.

When considering the NMs outlined within this review, it is evident

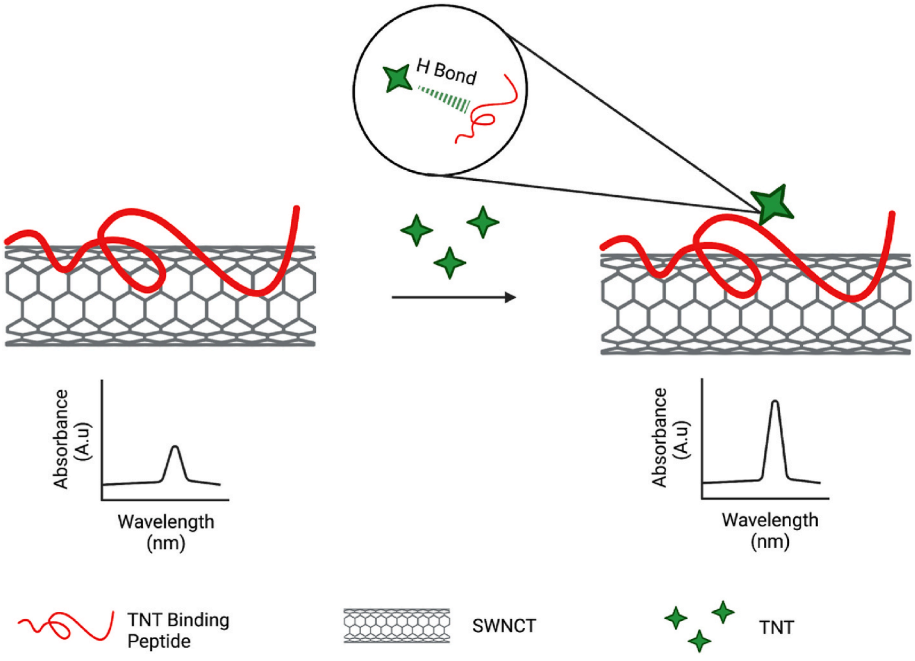

Fig. 8. NIR optical biosensor for the detection of TNT developed by Wang. A TNT-binding peptide is anchored to the side of the SWNT via π-π stacking interactions. Upon binding to TNT molecules, an increase in the absorption value in the near-infrared spectrum is seen, indicating a positive target interaction. Figure adapted with permission from Ref. [126].

that many forms of NMs are suitable for incorporation in optical biosensing platforms, with each possessing its specific advantages. Qdots appear to be a promising choice for use within forensic biosensors due to their superior optical properties, which produce high brightness, good photostability and a broad absorption spectrum [140]. The use of Qdots with various biological sensing moieties such as antibodies and enzymes has been demonstrated, resulting in biosensors with low LODs and no known interference with sample integrity, which is vital for forensic analysis. The mechanisms outlined within this review have been highlighted as biosensor mechanisms that can be adapted towards a range of targets within forensic analysis. When considering the toxicity associated with Qdots, providing that appropriate safety measures are considered alongside their use, there is no issue with employing a Qdot-based biosensor into a forensic workflow. Future research could aim to investigate novel nanomaterials, such as polymer nanoparticles, which are emerging as suitable conjugates to biosensors due to their range of advantages over the commonly used NMs mentioned within this literature. PNPs are a form of fluorescent NPs that are often used within sub-fluorophore groups and have shown great potential as novel fluorogenic substrates for biosensors [141]. These novel NPs are non-toxic and possess good stability, rapid emission rate and increased fluorescent brightness. Compared to the NMs outlined within this review, polymer NPs are an inexpensive option and have a rapid synthesis that is ideal for the high-throughput requirement of a forensic biosensor [142]. When considering a NM for inclusion within an optical biosensor, the NM and biosensing platform used will be dependent on the target analyte chosen. When designing an optical biosensor it is crucial to consider factors such as the sampling matrix, the desired optical signal and the personnel who will be operating the biosensor. This can help to guide an appropriate detection mechanism that is tailored to the analytical purpose.

Prior to the implementation of novel forensic biosensors into the workflow, optimisation of the sensitivity and specificity of the designed biosensor is crucial, as well as validation studies to determine the LOD, potential sources of cross-reactivity as well as stability studies. These studies are vital for all branches of forensic science as cross-reactivity cannot be accepted in order to achieve reliable and accurate results. This is especially important for bodily fluid detection and illicit drug screening where evidence may be relied upon in court and influence the deliverance of justice. Exploring numerous optical biosensing platforms such as FRET, bioluminescent resonance energy transfer, fluorescent-based and surface plasmon resonance-based to determine the most effective signal transduction is important to ensure that a biosensor is optimised for use in situ. In conclusion, this review has outlined numerous biosensor designs and mechanisms for forensic analysis that show great promise to provide sensitive and specific detection of forensically relevant target analytes. The vast potential for introducing biosensors into the forensic workflow is not something to be overlooked. As research in the field continues to expand there is no doubt that portable biosensors for the detection of illicit drugs, biological traces and environmental contaminants will become universal within forensic analysis and will streamline the forensic workflow.

Author statement

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Data availability

No data was used for the research described in the article.

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